

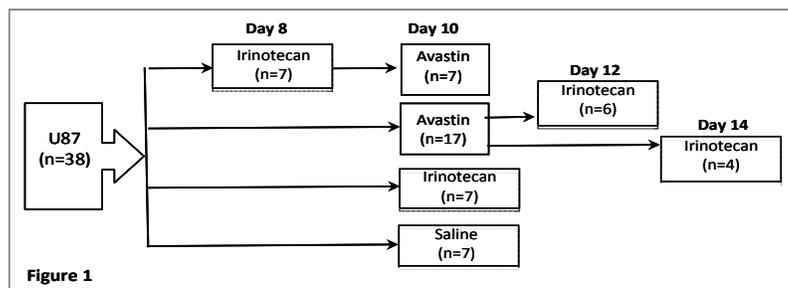
# Optimization of Combined Bevacizumab Plus Irinotecan Therapy in Brain Tumors Using MRI Measures of Relative Cerebral Blood Volume

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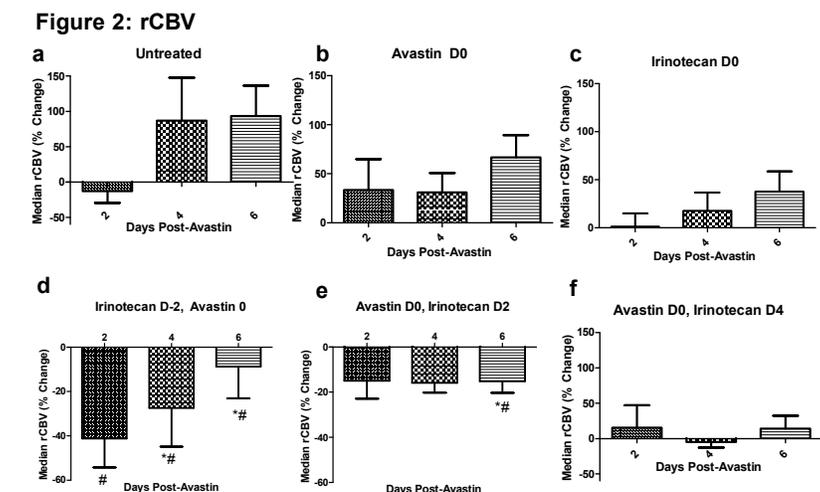
**Introduction:** The anti-angiogenic drug bevacizumab has recently been approved by the US Food and Drug Administration for the treatment of recurrent glioblastoma multiforme, the most common and most aggressive primary brain tumor. Frequently, bevacizumab is combined with a chemotherapeutic such as irinotecan, an approach motivated by studies that showed improved clinical outcomes compared to historical controls<sup>1</sup>. However, no systematic studies have been performed to determine if and how these drugs should be combined for optimal therapeutic response. Furthermore, it is becoming increasingly clear that standard MRI measures of response, which entail measuring contrast-enhanced tumor volume, are not appropriate for the evaluation of anti-angiogenic drugs since these drugs also decrease contrast extravasation. Consequently, the goal of this study was to demonstrate the utility of using rCBV (relative cerebral blood volume), derived from DSC imaging, to evaluate and optimize the combination of bevacizumab and irinotecan in the treatment of a U87 xenograft brain tumor model.

**Methods: Animal Model:** U87 human grade III astrocytoma cells were cultured, harvested, and 200,000 cells were injected into athymic rats (n=38) using intracranial, stereotaxic approach. The temporal treatment paradigm is described in Figure 1 where bevacizumab (Avastin, Genentech, South San Francisco, CA), given at day 10 (5 mg/kg), was combined with Irinotecan (125 mg/m<sup>2</sup>) (Camptosar, Pfizer, New York, NY) administered at days 8, 12 and 14 or alone at day 10 post-inoculation. Imaging was performed on days 10, 12, 14, and 16, which are days 0, 2, 4 and 6 post-Avastin. **MRI:** Images were obtained on a Bruker 9.4 T scanner fitted with a linear transmit coil, and surface receive coil. For DCE, 5 sets of SPGR pre-contrast images were acquired at five different flip angles ( $\alpha = 2^\circ\text{-}35^\circ$ ) to measure pre-contrast T1, with TE/TR= 1.892ms/46.875 ms; FOV= 4cm; matrix= 96. Next dynamic images were obtained with  $\alpha = 35^\circ$ , and gadodiamide (0.1 mmol/kg Omniscan, Nycomed Amersham) injected at the ninth acquisition. This also served as a loading dose for DSC imaging. DSC images were acquired using an EPI sequence (TE/TR=18ms/1s, FOV=3.5cm, matrix=96). Post contrast T1 weighted images were acquired using a spin-echo T1-weighted RARE sequence (TE/TR= 12.6ms/1500ms, FOV =3.5cm, matrix =256).



**Analysis:** The vascular permeability factor,  $K^{trans}$ , was determined from the DCE data.<sup>2</sup> The DSC data was processed to create rCBV maps, corrected for any leakage effects<sup>3</sup>. Enhancing tumor volumes (reported in mm<sup>3</sup>) were determined from the post-contrast T1w images, in all slices showing enhancing tumor. A two-tailed Mann-Whitney test was used to determine if significant differences existed between the untreated group and each of the treated groups at each post-treatment day. An  $\alpha=0.05$  level of significance was used.

**Results:** Figure 2 shows the median rCBV as a function of three treatment days for the different treatment paradigms. Treatment with either Avastin (Fig 2b) or Irinotecan alone (Fig 2c) shows an inhibition of angiogenesis compared to the control (untreated) case (Fig 2a). However, in cases for which irinotecan is combined 2 days before or 2 after treatment with Avastin (Fig 2d,e) there is a significant inhibition of tumor angiogenesis compared to both the untreated and Avastin monotherapy conditions. Avastin treatment at day 0 inhibited tumor growth as measured by enhancing tumor volume on days 2, 4, and 6 except in animals treated with irinotecan at day -2 (not shown). Rats treated with irinotecan alone showed no significant difference in tumor volume compared to controls. No difference in  $K^{trans}$  resulted in response to treatment with Avastin at d0 compared to untreated animals.



**Discussion:** The degree of tumor vascularity in the U87MG xenograft model depends on the timing of the irinotecan and Avastin therapies. Specifically, irinotecan administered within 2 days of Avastin resulted in the maximal decrease in tumor rCBV. Inhibition of tumor volume demonstrated different trends from tumor vascularity. However, in these studies, unlike the clinical population, treatment with Avastin does not eliminate enhancing tumor volume or significantly decrease  $K^{trans}$ . This phenomenon may be explained by the presence of rodent VEGF, as Avastin only binds to human VEGF produced by the human tumor cells. Combination therapies with blockade of human and rodent VEGF will be studied in subsequent rCBV studies using our xenograft model and incorporation of radiation and other chemotherapeutics. Our results demonstrate that rCBV is useful in determining the optimal temporal paradigms in preclinical studies and may likewise assist in more effective design treatment paradigms for malignant gliomas in patients.

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**References** (1) Zuniga RM et al. J Neuro-Onc 2009; 97:950-951. (2) Tofts et al. MRM 1991; 17:357-367. (3) Schmainda KM et al. Am J Neuroradiol 2004; 25:1524-1532.